

WHAT IS CLAIMED IS:

1. An antibody that specifically binds to AC133 antigen.
- 5 2. An antibody according to Claim 1, wherein said antibody is a monoclonal antibody produced by a hybridoma cell line.
3. A monoclonal antibody according to Claim 2, wherein said antibody blocks simultaneous binding to said antigen by the antibody produced by the hybridoma cell line  
10 ATCC .
4. A monoclonal antibody according to Claim 1, wherein said antibody is induced through contralateral immunization.
- 15 5. A monoclonal antibody according to Claim 1, produced by the hybridoma cell line ATCC \_\_\_\_.
6. A method for enrichment of hematopoietic stem or progenitor cells or both, said method comprising:  
20 combining a mixed population of human cells comprising hematopoietic stem or progenitor cells or both with a reagent that specifically binds to the hematopoietic progenitor cell antigen recognized by the antibody produced by the hybridoma cell line ATCC \_\_\_\_; and  
selecting for those cells that bind said reagent;  
25 wherein said selected cells are enriched in hematopoietic stem or progenitor cell activity or both, depending on whether said mixed population of human cells contained hematopoietic stem or progenitor cells or both, respectively.
7. A method according to Claim 6, further comprising:  
30 combining said mixed population of human cells with a reagent that

specifically recognize at least one of the cell surface markers CD90, CD117 and HLA-DR;  
and

selecting for those cells that are positive for said at least one of said cell  
surface markers.

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8. A method according to Claim 6, wherein said reagent is an antibody or an  
antibody mixture.

9. A method according to Claim 8, wherein at least one of said antibodies is  
10 fluorochrome conjugated.

10. A method according to Claim 9, wherein said selecting with said  
fluorochrome conjugated antibodies is by flow cytometry.

11. A method according to Claim 8, wherein at least one of said antibodies is  
15 conjugated to magnetic particles.

12. A method according to Claim 11, wherein said selecting with said magnetic  
particle conjugated antibodies is by high gradient magnetic selection.

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13. A substantially pure population of hematopoietic progenitor cells, wherein  
said cells are bound to a reagent that specifically binds to the hematopoietic progenitor cell  
antigen recognized by the antibody produced by the hybridoma cell line ATCC HB12346.

14. A substantially pure population of hematopoietic progenitor cells according to  
25 Claim 13, wherein said reagent is a monoclonal antibody.

15. A substantially pure population of hematopoietic progenitor cells according to  
Claim 14, wherein said monoclonal antibody is produced by the hybridoma cell line ATCC  
30 HB12346.

16. A substantially pure population of hematopoietic progenitor cells according to Claim 15, wherein said progenitor cells are obtained from human fetal liver.

5 17. A substantially pure population of hematopoietic progenitor cells according to Claim 15, wherein said progenitor cells are obtained from human peripheral blood.

18. A substantially pure population of hematopoietic progenitor cells according to Claim 15, wherein said progenitor cells are obtained from human bone marrow.

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19. A substantially pure population of hematopoietic progenitor cells according to Claim 18, wherein said bone marrow is adult.

20. A substantially pure population of hematopoietic progenitor cells according to  
15 Claim 18, wherein said bone marrow is fetal.

21. An isolated nucleic acid molecule, wherein said molecule comprises:

(1) a first sequence having an amino acid coding region for AC133 as set forth in Figure 12 (SEQ ID NO:1);

20 (2) a second sequence, wherein said second sequence is a subsequence of said first sequence and is at least 14 nucleotides in length;

(3) a third sequence in which at least one nucleotide of said first or second sequences is replaced by a different nucleotide; or

(4) a fourth sequence complementary to any of said first, second or third  
25 sequences;

with the proviso that (i) if said molecule is an RNA molecule, U replaces T in said sequence of said molecule, (ii) said third sequence is at least 90% identical to said first or second sequence, and (iii) said second sequence is not nucleotides 347-667, 1564-1696, or 2010-2386 of SEQ ID NO:1.

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22. The isolated molecule of claim 21, wherein said molecule comprises said first sequence.

23. The isolated molecule of claim 21, wherein said molecule comprises said  
5 second sequence.

24. The isolated molecule of claim 21, wherein said molecule comprises said third sequence.

10 25. The isolated molecule of claim 21, wherein said molecule consists essentially of DNA encoding the amino acid sequence of AC133.

26. An expression vector comprising a nucleic acid sequence of claim 21.

15 27. A cell transfected with the molecule of claim 26.

28. An isolated polypeptide, wherein said polypeptide comprises: (1) a first amino acid sequence of AC133 as set forth in SEQ ID NO: 2; (2) a second amino acid sequence wherein said second sequence is a subsequence of said first sequences and is at least 6 amino  
20 acids in length; or (3) a third sequence in which at least one amino acid of said first or second sequences is replaced by a different amino acid, with the proviso that said amino acid replacement is a replacement of one acidic residue for another, one basic residue for another, one non-polar residue for another, one uncharged polar residue for another, or one aromatic residue for another, with the proviso that said third sequence is at least 90% identical to said  
25 first or second sequence.

29. The isolated polypeptide of claim 28, wherein said polypeptide comprises said first sequence.

30 30. The isolated polypeptide of claim 28, wherein said polypeptide comprises said

second sequence.

31. The isolated polypeptide of claim 28, wherein said polypeptide comprises said third sequence.

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32. The polypeptide of claim 29 complexed to a ligand.

33. The polypeptide complex of claim 32, wherein said ligand is an antibody.

10 34. An isolated polypeptide, wherein said polypeptide comprises the amino acid sequence from extracellular N-terminus, aa 20-107; first transmembrane region, aa 107-126; first cytoplasmic loop, aa 127-157; second transmembrane region, aa 158-179; first extracellular loop, aa 180-435; third transmembrane region, aa 436-454; second cytoplasmic loop, aa 455-480; fourth transmembrane region, aa 481-503; second extracellular loop, aa  
15 504-792; fifth transmembrane, aa 793-816; or cytoplasmic C-terminus, aa 817-865; of SEQ ID NO:2.

35. A method for identifying a ligand that binds to human hematopoietic stem cells, comprising detecting binding of said ligand with the polypeptide of claim 8, .

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36. A reagent that specifically binds to the polypeptide of claim 28.

37. The reagent of claim 36, wherein said reagent is selected from the group consisting of monoclonal and polyclonal antibodies.

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38. The reagent of claim 36, wherein said reagent is a physiological or synthetic ligand.

39. The polypeptide of claim 28, wherein said polypeptide is not glycosylated.

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40. The polypeptide of claim 28, wherein said polypeptide is glycosylated.

41. In a method of isolating hematopoietic stem cells using a cell separation technique based on identification of stem cells by selective binding of a ligand to an  
5 antigenic marker on said stem cell, an improvement which comprises:  
utilizing an AC133 antigen as said antigenic marker.

42. The method of claim 41, wherein said ligand is an antibody.

10 43. The method of claim 41, wherein said ligand binds to an extracellular region  
of said AC133 antigen.

44. The method of claim 41, wherein said extracellular region comprises an amino  
acid selected from extracellular N-terminus, aa 20-107; first extracellular loop, aa 180-435;  
15 or second extracellular loop, aa 504-792; of SEQ ID NO:2.

45. The method of claim 41, wherein said ligand has been identified by  
determining whether compounds in a group of test compounds bind to said AC133 antigen  
and selecting said ligand from among compounds that bind specifically to said AC133  
20 antigen with less than 10% crossreactivity with any antigen present on mature blood cells.

46. The method of claim 41, wherein crossreactivity is measured by a competitive  
binding assay between pure AC133 antigen, said ligand, and said suspected crossreactive  
antigen using concentrations of AC133 antigen and said ligand where said ligand half-  
25 saturates binding to AC133.

47. The method of claim 41, wherein crossreactivity is measured at a  
concentration of AC133 antigen that half saturates monoclonal antibody ATCC HB12346  
when said antibody is present at a concentration of 50 ng/100  $\mu$ l.

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48. A ligand for AC133 identified by the method of claim 36.

49. A reagent that bind specifically to AC133 antigen with less than 5% crossreactivity with any antigen present on mature blood cells.

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50. The reagent of claim 49, wherein said reagent is attached to a surface or to a detectible label.

51. The reagent of claim 49, wherein said label is a fluorescent label.

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